

Natural killer cells in infection and inflammation of the lung

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doi:10.1111/j.1365-2567.2009.03167.x

Received 20 July 2009; accepted 21 July 2009.

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Summary

The lungs are a major site of entry of pathogens into the body and thus require rapid and effective innate responses to prevent pathogens establishing infection and to limit their spread. Additionally, the immune response in the lung must be tightly regulated such that pathogens are cleared, but immunopathology and chronic inflammation are prevented. In this review, I consider the role of natural killer (NK) cells in pulmonary infection and inflammation, specifically their contributions to influenza, tuberculosis, asthma and chronic obstructive pulmonary disease (COPD), which are major causes of morbidity and mortality world-wide. Despite evidence of the importance of NK cells in these diseases, there are still major gaps in our understanding of how their function is regulated in this unique tissue environment. Understanding how different beneficial and detrimental effector functions of NK cells are triggered will be crucial if NK cells are to be exploited therapeutically in respiratory disease.

Keywords: infection; inflammation; lung immunology/disease

Introduction: natural killer cells in the lung

Natural killer (NK) cells are innate lymphocytes which are a first line of defence against infection and cancer.^{1,2} NK cells form synapses with diseased cells, but also other leucocytes, including macrophages and dendritic cells, in which they integrate activating and inhibitory signals from a multitude of germline-encoded receptors.^{3–7} Activating receptors include the natural cytotoxicity receptors (NCRs), such as NKp46 and NKp44, the Fc receptor CD16 and NKG2D.⁷ The ligands for NK cell-activating receptors include both host and pathogen glycoproteins; for example, NKG2D recognizes the stressed-induced ligand MHC class I polypeptide-related sequence A (MICA).^{8,9} Inhibitory receptors, such as killer immunoglobulin-like receptors (KIRs) and the NKG2A:CD94 dimer, generally recognize classical and non-classical class I major histocompatibility complex (MHC) molecules, and NK cell activation can also be triggered by loss of inhibitory ligands from the cell surface.^{10,11} In addition, NK cells are activated by cytokines, including type I interferons, interleukin (IL)-12 and IL-18.^{12–14} Once activated, NK cells can direct cytolytic granules towards the synapse to directly kill a target cell.^{1,15} Our understanding of NK cells is evolving rapidly and their functions clearly go beyond those of innate killer cells. Importantly, NK cells are a potent and

early source of cytokines, particularly interferon (IFN)- γ , but they can also produce T helper type 2 (Th2)-associated cytokines, such as IL-5 and IL-13, and the regulatory cytokine IL-10.¹⁶ NK cells also specialize their function at different tissue locations: recently, a novel IL-22-secreting subset of NK cells has been described in the gut and tonsils.^{17–19} The interrelationships and functions of different NK cell subsets are not fully understood, but in humans, NK cells expressing high levels of CD56, the predominant subset in lymph nodes, exhibit higher cytokine production but diminished cytotoxicity relative to CD56 dim cells, which are the major subset in the periphery.²⁰ NK cells can be activated by interactions with dendritic cells and macrophages and profoundly influence the generation of the adaptive response.^{1,2,21–23} The existence of memory in NK cells, that is long-term alteration of NK cell responses according to previous experience, has also been recently described.^{24–26}

Here, I review the contribution of NK cells to respiratory infections and inflammatory disorders of the lung. The airways are a major route of entry of many important pathogens into the body and the ability of NK cells to respond rapidly to infection suggests an important role for these cells in acute pulmonary infection. However, evidence is emerging that NK cells are also important in regulating chronic infection and inflammation, and thus

may play important roles in chronic infections, such as tuberculosis, and chronic inflammatory disorders of the airways, such as asthma.

NK cells make up 10% of resident lymphocytes in the lung, in numbers second only to those in the spleen,^{27–29} and their survival may be promoted by bronchial epithelial cells which spontaneously produce IL-15.³⁰ Within days of infection, or hours after inflammatory stimulation, large numbers of NK cells are recruited to the lung from the blood and become activated to secrete cytokines, particularly IFN- γ .^{28,31–35} The airways are a unique environment in which the immune response must function. In homeostasis, the upper airways must tolerate continuous exposure to environmental antigens and commensal organisms. During infection, innate responses in the lung must be induced rapidly, but inflammation must be balanced to avoid damage to airway structures and airway occlusion, leading to impaired gaseous exchange. Inflammation in the lung is restrained, chiefly by IL-10 and transforming growth factor (TGF)- β produced by alveolar macrophages, which raise the threshold of activation which needs to be overcome before immune responses can occur.³⁶ In homeostasis, pulmonary NK cells from bronchoalveolar lavage (BAL) or from lung tissue are suppressed; they can form conjugates with target cells, but are profoundly impaired in their cytotoxic capacity.^{29,37,38} Lung NK cells regain their activity after 24 hr in culture or stimulation with type I IFN, and, conversely, peripheral blood NK cells can be suppressed by culture with BAL fluid or alveolar macrophages, an effect unique to this type of macrophage.^{38–41} Soluble factors present in the lung that can regulate NK cell activity include TGF- β ,⁴² prostaglandins produced by alveolar macrophages^{28,43} and pulmonary surfactant.⁴⁴ Human leucocyte antigen (HLA)-G has also been reported to be expressed on pulmonary macrophages and dendritic cells during lung cancer; however, the role of HLA-G in regulating pulmonary NK cells during inflammation is unknown.⁴⁵ The importance of regulation of NK cells in the lung is illustrated by the fatal lung pathology caused when NK cells are systemically activated by exogenous IL-18 and IL-2.⁴⁶ Thus the extent of NK cell activation in the lung will depend on the balance of pro-inflammatory and regulatory factors.

Genetic deficiencies that effect NK cell function are rare, but have important implications for pulmonary health. In transporters associated with antigen processing-2 (TAP2)-deficient patients, class I MHC expression is defective and NK cells are poorly regulated. Early in life, NK cells are believed to protect patients against infection in the absence of effective T-cell immunity; however, later in life chronically activated NK cells are recruited to the skin and respiratory tract via chemokine (C-C motif) receptor 2 (CCR2), where they form lethal granulomatous lesions.^{47–49} Furthermore, genetic defi-

ciencies that result in loss of NK cell function are associated with recurrent viral and bacterial infections, including those of the upper and lower respiratory tract.^{50–55} Next, I discuss our current state of knowledge of the role of NK cells in the acute respiratory viral infection influenza and the chronic bacterial infection tuberculosis. I also discuss the role of NK cells in the inflammatory disorders asthma, chronic obstructive pulmonary disease (COPD) and other cases of fibrosing airway disease.

NK cells in influenza infection

There is an urgent need for a better understanding of the immune response to influenza, with the goals of reducing pathology during infection and enhancing protection by vaccination.⁵⁶ The adaptive immune response, particularly that involving cytotoxic T cells and antibody, can protect against influenza.^{57,58} The cytotoxic lymphocyte response must be sufficiently rigorous to aid clearance of the virus, as illustrated by cases of severe influenza infection in infants characterized by a deficiency of NK and cytotoxic T lymphocytes in the lung,^{59,60} but dysregulation of the innate response results in a 'cytokine storm' and correlates with severity of symptoms.^{61–65}

NK cells are recruited to the lung within the first few days of influenza infection in humans and in murine models^{28,66} and depletion of lung NK cells leads to increased morbidity and mortality, within days of infection.^{35,67,68} NK cells reciprocally regulate the adaptive response in influenza: NK cells are required for activation of the cytotoxic T lymphocyte (CTL) response⁶⁹ and T-cell IL-2 production augments NK cell IFN- γ production in recall responses.⁷⁰

NKp46 is a key activating receptor which is critical for protecting mice against lethal influenza infection,⁷¹ and is one of the few known examples of direct binding of viral glycoprotein to an NK cell-activating receptor. Influenza haemagglutinin (HA) binds to both NKp46 and NKp44, largely via the α -2,6-linked terminal sialic acid, which is present on residue Thr225 of NKp46.^{72–74} The ability of NK cells to be activated by different influenza strains is influenced by levels of HA expression, HA affinity for sialic acid and HA glycosylation.^{73,75,76} However, it is not clear how the specificity of this interaction is conferred, that is, why other related receptors such as NKp30, which are likely to be similarly glycosylated, do not exhibit the same interaction with HA. NK cells are activated by influenza-infected monocytes and dendritic cells, via both contact-dependent mechanisms and cytokines.⁷⁷ Enhanced cytotoxicity of NK cells is stimulated by IFN- α secretion; CD69 up-regulation is induced by IFN- α , NKG2D recognition of the ligands UL16-binding protein (ULBP)1–3, and NKp46 ligation of HA, and IFN- γ secretion is stimulated by IL-12, NKG2D and NKp46.⁷⁹

To counter recognition by NK cells, influenza causes reorganization of MHC I into aggregates within GM1 ganglioside (GM-1) rich lipid microdomains, which increases binding of the inhibitory receptors KIR2DL1 and LIR, increasing inhibition of NK cell function.^{80,81} Responses of NK cells to influenza-infected monocytes were dependent on the KIR/HLA compound genotype, providing evidence that KIR/HLA-C interactions have a significant role in cytotoxicity and represent a mechanism by which these genotypes may influence influenza and other viral infections.⁸²

Antibodies to influenza matrix protein 2 (M2), which is expressed on the surface of infected cells, required NK cells to confer protection *in vivo*, suggesting that, once an antibody response to influenza has developed, antibody-dependent cell-mediated cellular cytotoxicity (ADCC) is mediated by NK cells and contributes to viral clearance.⁸³ NK cells express tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) early in influenza infection, but blockade of TRAIL unexpectedly decreased viral titre at this time-point.⁸⁴ The role of IL-18 in influenza infection is controversial. One group reported that, in the absence of IL-18, NK cell activity and IFN- γ production were reduced and early viral replication was poorly controlled,⁸⁵ whereas others reported decreased viral load, with no difference in pathology or NK cell IFN- γ production in the same IL-18-deficient mice.⁸⁶

In conclusion, during influenza infection, NK cells are recruited to the lung where they could potentially interact with virally infected epithelial cells, monocytes, dendritic cells and T cells (Fig. 1). They contribute to protection against influenza, limiting early viral replication and promoting an effective CTL response. Yet, the mechanisms involved in achieving this, for example the relative importance of NK cell cytokine production versus cytotoxicity, over the time-course of influenza infection are unclear.

NK cells in tuberculosis

One third of the world's population are currently infected with *Mycobacterium tuberculosis* (MTb), and this infection results in almost 2 million deaths annually.⁸⁷ In the majority of people, the infection remains in a chronic latent state, in which the immune response prevents bacterial dissemination, but is not so vigorous as to cause immunopathology. Mycobacteria survive within macrophages, which can kill the bacteria if sufficiently activated, so induction of a Th1-type response, and in particular IFN- γ production, is key to protection against infection.^{88,89} The importance of the innate response in disease is still unclear.⁹⁰

NK cell NKp46 expression and cytotoxicity are reduced in freshly isolated peripheral blood mononuclear cells (PBMCs) from tuberculosis patients, which may be attributable to suppression by monocytes and IL-10.^{91–93} NK

cells in the pleural effusion, the excess fluid that collects around the lungs of patients with tuberculosis, are enriched for CD56hi cells with reduced expression of CD16 and perforin, which may be attributable to selective apoptosis of CD56dim cells induced by as yet unidentified soluble factors in pleural fluid.⁹⁴ In accordance with the CD56hi subset of NK cells being associated with high cytokine production, NK cells from pleural effusions spontaneously produced IFN- γ and responded strongly to re-exposure to MTb by producing IFN- γ , and this IFN- γ production correlated with disease severity.⁹⁴ Thus, in active disease, NK cells exhibit reduced cytotoxicity but increased IFN- γ production, perhaps because of selective activation of NK cell subsets.

Human NK cells can be activated by and induce apoptosis in mycobacteria-infected monocytes and macrophages *in vitro*,^{95,96} mediated by NKp46 recognition of vimentin and NKG2D recognition of its ligand ULBP-1.^{93,97,98} NK cells can also be activated by direct binding of NKp44 to the mycobacterial cell wall, although the ligand remains undetermined.^{99,100} MICA is the gene most strongly associated with susceptibility to the opportunistic *Mycobacterium avium* and is expressed in the epithelium, macrophages, epitheloid cells and multinucleated giant cells in infected tissues, suggesting a potential role for this NKG2D ligand in mycobacterial infection.¹⁰¹ As well as direct killing of infected cells, NK cells may also regulate the T-cell response to MTb. In mixed PBMC cultures stimulated with MTb, NK cell IFN- γ production and CD40:CD40L interactions with infected monocytes stimulated IL-15 and IL-18 production by monocytes and promoted expansion and cytotoxicity of CD8⁺ cells.¹⁰² In similar mixed cultures, NK cells lysed activated regulatory T cells (Tregs) via NKp46 and NKG2D:ULBP1 interactions.¹⁰³ Thus, overall, many cell types express ligands that could activate NK cells in the lung during mycobacterial infection (Fig. 1).

Are NK cells important in MTb infection *in vivo*? Animal models do not give a clear answer to this question. NK cells are activated and produce IFN- γ in the lung following mycobacterial infection.^{104–107} In T-cell-deficient mice, a protective role for IL-12-induced IFN- γ production by NK cells has been demonstrated.¹⁰⁸ However, depletion of NK cells had no effect on bacterial replication in the lung of immunocompetent mice,¹⁰⁴ suggesting that NK cells may be redundant in the presence of intact adaptive immunity. In fact, surprisingly, IFN- γ knockout (KO) mice, which are impaired in their ability to clear mycobacteria, cleared them as effectively as wild-type mice when NK cells were depleted, suggesting that NK cells can inhibit protective immunity.¹⁰⁵ It should be borne in mind that murine models may poorly reflect the situation in humans; for example, although lymphocyte aggregates form in the lung, the classical granuloma does not.¹⁰⁹

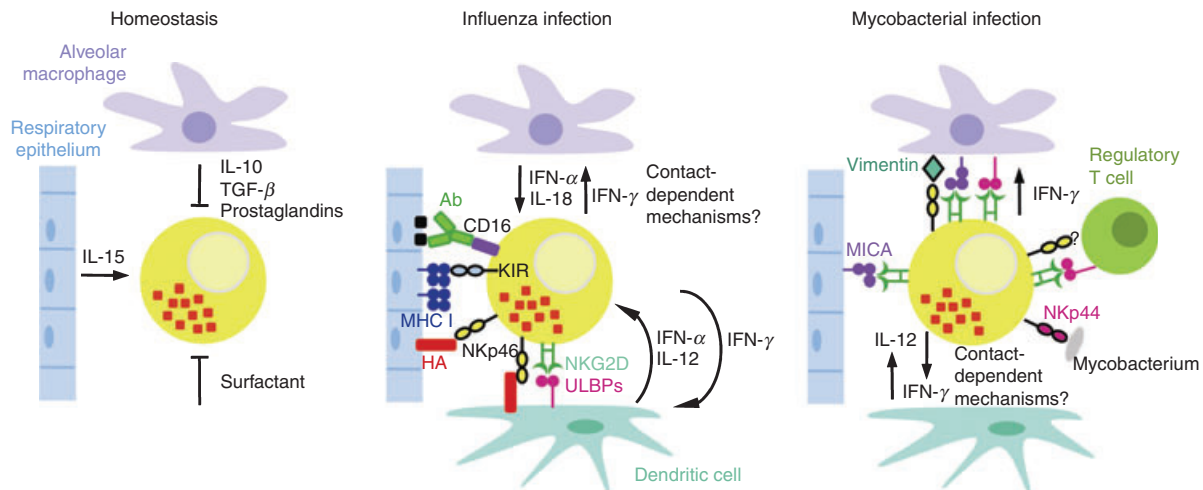


Figure 1. Potential activating and inhibitory interactions of natural killer (NK) cells in the lung. NK cell function in the lung is regulated by both contact-dependent interactions and soluble mediators. Note that, although many of these interactions have been demonstrated *in vitro*, their timing, location and relative importance *in vivo* are not known. HA, haemagglutinin; IFN, interferon; IL, interleukin; KIR, killer immunoglobulin-like receptor; MICA, MHC class I polypeptide-related sequence A; MHC, major histocompatibility complex; TGF, transforming growth factor; ULBP, UL16-binding protein.

To reconcile the data and understand the importance of NK cells in MTb infection it may be necessary to differentiate their contributions at different stages of disease. Recently, it was shown that during chronic infection with *Leishmania donovani*, another pathogen that inhabits macrophages, NK cells are recruited to liver granulomas where they produce IL-10, which suppresses cell-mediated immunity.¹⁶ Such a mechanism may explain the apparent suppressive role of NK cells observed in the murine model of mycobacterial infection.¹⁰⁵ Thus, to fully understand the role of NK cells in tuberculosis, it may be necessary to define their roles in limiting early infection, inducing protective adaptive immunity and maintaining latency, and during re-activation of infection.⁹⁰

Other murine models of pulmonary infection

The contribution of NK cells to a number of other pulmonary infections has been studied in murine models (Table 1). The requirement for NK cells in respiratory infection and inflammation can be demonstrated by depletion, but cases where this is the only evidence for NK cell involvement in infection must be interpreted with caution, as the commonly used markers for depletion, NK1.1 and asialo-GM1, are also expressed on other lymphocyte subsets.

NK cells in asthma

300 million people world-wide suffer from asthma, which in the majority of cases is associated with allergy to environmental antigens.¹¹⁰ Acute attacks caused by allergen exposure trigger mast cell degranulation, eosinophilic

inflammation, mucus production and bronchoconstriction. In the long term, airway remodelling, characterized by airway thickening caused by extracellular matrix deposition, and muscle and goblet cell hypertrophy, results in diminished airway function.¹¹¹ Inflammation and pathology in asthma are driven by the production of Th2 cytokines (IL-4, IL-5, IL-13, IL-9 and IL-3), which have pleiotropic effects on leucocytes and airway stromal cells.

NK activity is enhanced in PBMCs from asthmatics.^{112–114} Immediately after allergen challenge, this activity declines, consistent with extravasation of NK cells to the lung.¹¹² This is also observed in an animal model of allergic airway sensitization,³² and could result from the release of chemo-attractants by activated mast cells.¹¹⁵ In contrast, an increased frequency of NK cells was reported in PBMCs from asthmatic children during acute exacerbations, which resolved when children were in a stable condition.¹¹⁶ However, whether these exacerbations were caused by viral infection was not determined. The phenotype of NK cells is also altered in asthma and allergy. Atopic asthmatics were reported to have a slightly higher frequency of IL-4⁺ and a lower frequency of IFN-γ⁺ NK cells following *ex vivo* activation of PBMCs,^{117,118} and purified peripheral NK cells of patients with atopic dermatitis spontaneously released high amounts of IFN-γ, IL-4, IL-5 and IL-13.¹¹⁹ Thus NK cells may contribute to the balance of Th1 and Th2 cytokines in asthma and allergy.

The mechanisms by which NK cells are stimulated to produce different cytokines are poorly understood. Human and mouse NK cells produce IL-5 and IL-13 (and in some cases IL-4) when activated *ex vivo*, and production of these cytokines is selectively promoted by IL-4, and inhibited by IL-12 or IL-10.^{120–129} In freshly isolated

Table 1. The role of natural killer (NK) cells in murine models of pulmonary infection

Pathogen	Protective effect of NK cells?	Possible protective functions of NK cells	Proposed mechanism of NK cell activation	Notes	References
Fungi					
<i>Cryptococcus neoformans</i>	Yes – promote clearance	IFN- γ production	IL-18	NK cells are a major source of IFN- γ in IL-12 ^{-/-} mice	176
<i>Aspergillus fumigatus</i>	Yes – promote survival and clearance of pathogen	IFN- γ enhances macrophage fungicidal activity and induces chemokine production in the lung		Opportunistic. NK cell IFN- γ is sufficient to mediate clearance	33,177
Bacteria					
<i>Bordetella pertussis</i>	Yes – promote bacterial clearance	IFN- γ activates macrophages and suppresses Th2 response	Production of IL-12 by activated DCs		178
<i>Streptococcus pneumoniae</i>	No – detrimental effect on clearance of bacteria	Major source of IFN- γ	Pneumolysin activated monocytes	Experiments performed in scid ^{-/-} mice	179
<i>Francisella tularensis</i>	Yes – promote survival	Early source of IFN- γ promotes clearance of bacteria and Th1 responses			180
<i>Legionella pneumophila</i>	Yes – mediate pathogen clearance	Early source of IFN- γ	NK cell activation is dependent on MyD88 in NK cells		181
<i>Haemophilus influenzae</i>	Yes – required for pathogen killing	Stimulate killing of intracellular bacteria by PMNs	Activation requires IL-15 production by Gr-1hi PMNs		182
<i>Pseudomonas aeruginosa</i>	Yes – critical for bacterial clearance	IFN- γ production	NKG2D	Opportunistic	173,183
<i>Staphylococcus aureus</i>	Yes	IFN- γ and TNF production, augmentation of phagocytosis by macrophages	Activation by infected macrophages and bacterial superantigen	Opportunistic	184–186
Viruses					
Herpes simplex virus (HSV)	Yes – mediate viral clearance	IFN- γ secretion and cytotoxicity	NK cell activation is IL-18, but not IL-12, dependent	HSV can cause pneumonia in neonates and immune-compromised patients	187,188
Respiratory syncytial virus (RSV)	Yes – viral clearance	Early IFN- γ secretion	Recruitment to the lung depends on macrophages		135,150,189,190

DC, dendritic cell; IFN, interferon; IL, interleukin; PMN, polymorphonuclear cell; scid, severe combined immunodeficiency; Th, T helper; TNF, tumour necrosis factor.

peripheral blood NK cells, IL-13 is predominantly produced by the CD56hi subset.¹²⁰ It has been proposed that cytokine production correlates with NK cell maturation, as culture of immature NK cells with IL-12 results in an irreversible change from IL-5 to IFN- γ production.^{130–134} So, the phenotype of NK cells in asthma and allergy could be a result of exposure to a Th2 cytokine environment. In support of this hypothesis, there is evidence that, in the lung, the cytokine profile of NK cells can be influenced by the nature of the T-cell response. In a murine model of respiratory syncytial virus (RSV) infection, the propor-

tion of NK cells secreting IFN- γ was augmented during a Th1 response, but reduced in a Th2 response.¹³⁵ This may be a result of the direct actions of Th1-produced IFN- γ on the NK cell phenotype *in vivo*.¹²⁶ However, T cells are not required for activation of IL-13-producing NK cells^{126,136} and IL-4 can stimulate IFN- γ -producing NK cells,¹³⁷ suggesting that polarization of NK cells does not simply echo the T-cell cytokine milieu. Other factors that could influence the NK cell phenotype in the lung in asthma include Prostaglandin D₂ (PGD₂), which is produced predominantly by mast cells¹³⁸ and can potently

inhibit NK cell IFN- γ production and cytotoxicity.¹³⁹ Asthmatics are also deficient in type I IFN production, which could impact on NK cell activation, particularly during viral exacerbations of asthma.^{13,140,141}

The differential activation of NK cells in asthma may have important functional consequences because of their ability to influence the adaptive response. NK cells activated with IL-12 can kill immature dendritic cells and it has been proposed that, through 'dendritic cell editing' during an immune response, they remove dendritic cells which would otherwise promote Th2 responses or tolerance.²¹ NK cells activated with IL-4 do not perform this function, and may therefore promote T-cell anergy or Th2 responses.¹⁴² Supporting this hypothesis, in patients with rhinitis and asthma, the proportion of CD56hi NK cells was low, and IFN- γ production and dendritic cell maturation, following co-culture with NK cells, were impaired.¹⁴³ There may be other consequences of the altered NK cell response in asthma. NK cells from asthmatics also expressed more CD95 (Fas) and affected T-cell activation by cyclic AMP (cAMP),¹⁴⁴ and thus may directly influence the T-cell response. Asthma exacerbations are strongly associated with respiratory viral infections and asthmatics experience more severe and longer-lasting symptoms following infection.^{145,146} Inappropriate or poor activation of NK cells in asthma could enhance susceptibility to these infections. NK cells may also influence sensitizing antibody [immunoglobulin E (IgE)] production directly or indirectly.¹¹⁹

Mouse models support an important role for NK cells in allergic airway inflammation. In a model of allergen sensitization followed by airway challenge, depletion of NK cells inhibited the development of allergic pulmonary inflammation, dramatically decreasing eosinophil numbers in the lung and serum IgE.¹⁴⁷ In this model, NK cell depletion during sensitization was necessary to reduce allergic inflammation, suggesting that NK cells were required for initiation of the Th2 response, as has been demonstrated for some Th1 responses.^{22,23,148} Prior infection with bacteria can activate NK cells such that they inhibit allergic sensitization and subsequent respiratory inflammation,¹⁴⁹ and activation of NK cells with IL-12 during sensitization inhibited eosinophilia in a respiratory virus model of airway inflammation.¹⁵⁰ NK cells can also influence ongoing allergic inflammation. In peritoneal inflammation, NK cell depletion during allergen challenge could reduce eosinophilia and IL-5 production,¹⁵¹ and IFN- γ -secreting NK cells induced *in vivo* by IL-2 and IL-18 significantly suppressed airway hyper-responsiveness and eosinophilia after allergen sensitization.¹⁵²

Taken together, these studies suggest that NK cell function is altered in asthma, towards a Th2-cytokine-producing phenotype. NK cells can promote allergic airway inflammation during sensitization and ongoing inflammation, but stimulation of NK cells towards an IFN- γ -secret-

ing phenotype can reduce allergic airway pathology, at least in animal models. Our knowledge of the signals that stimulate different phenotypes of NK cell cytokine secretion in asthma and allergic responses is still very limited. Are NK cells already polarized and influencing dendritic cell and T-cell activation during sensitization? If so, what causes early differentiation of NK cells? How NK cells promote ongoing allergic sensitization and the relative importance of direct cytokine production, or interactions with T cells and accessory cells, are also areas that deserve further study.

NK cells in fibrotic lung disease

Pulmonary fibrosis occurs as a result of chronic lung inflammation, in diseases including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and idiopathic pulmonary fibrosis (IPF).¹⁵³ Persistent inflammation results in dysregulation of the normal wound healing responses, and generation of pro-fibrotic cytokines (IL-13 and TGF- β) and growth factors, leading to accumulation of extracellular matrix components, with resulting impairment of airway function. COPD is a chronic inflammation of the lung, the primary risk factor for which is cigarette smoking, which affects 210 million people world-wide.^{110,154} COPD is associated with destruction of the lung parenchyma (resulting in emphysema), and inflammation and obstructive fibrosis of the bronchioles. The immunological mechanisms underlying COPD are still poorly understood.¹⁵⁴ Idiopathic pulmonary fibrosis is the name given to fibrotic lung disease of unknown origin, which is generally fatal within 2–5 years and which is considered a Th2 disease.

NK cell function is impaired in COPD, which can be partially attributed to the effects of smoking, which reduces NK cell function in the lungs and peripheral blood,^{155–158} possibly by increasing the numbers of immunosuppressive alveolar macrophages.³⁹ However, peripheral blood NK cell cytotoxicity is reduced even in ex-smokers with COPD, compared with control ex-smokers, suggesting a deficiency associated with disease.^{159,160} In patients with IPF, expression of NKG2D was reduced on NK, NKT and $\gamma\delta$ cells in BAL, which may be a consequence of the increased expression of soluble MICA or TGF- β in these patients.^{161–163} Patients with IPF also strongly express MICA on epithelial cells and fibroblasts in the lung and have a significant increase in the frequency of the MICA*001 allele and a decrease in the frequency of MICA*004, suggesting that this NK cell ligand may play a role in regulating disease progression.¹⁶³

NK cells may mediate a protective effect against fibrosis. In models of bleomycin-induced pulmonary fibrosis, lack of NK cell recruitment, in the absence of chemokine (C-X-C motif) receptor 3 (CXCR3), resulted in an absence of IFN- γ in the lung and enhanced fibrosis, and

exogenous IFN- γ treatment had a therapeutic effect, demonstrating the importance of NK cell IFN- γ in regulating pulmonary fibrosis.^{164,165} However, although initial studies suggested that IFN- γ could be used therapeutically in patients with idiopathic pulmonary fibrosis,¹⁶⁶ a recent large multicentred trial did not find any clinical benefit of this treatment.¹⁶⁷

By what mechanisms, other than IFN- γ production, could NK cells protect against pulmonary fibrosis? In hepatic fibrosis, NK cells promote disease resolution by selective recognition and killing of collagen-secreting stellate cells.^{168–171} As the balance of fibroblast proliferation and apoptosis underlies the extent of pulmonary fibrosis,¹⁵³ it would be interesting to know if NK cells can also regulate numbers of collagen-secreting cells in the lung. Importantly, the ability of NK cells to protect against infection may also limit airway inflammation and consequently fibrosis. Respiratory infections are more prevalent in COPD and most exacerbations of COPD and asthma are caused by infections.^{146,172} In CF, chronic infections lead to lung fibrosis, and NK cells, activated via NKG2D, secrete IFN- γ which mediates clearance of the principal opportunistic infection in CF, *Pseudomonas aeruginosa*.¹⁷³ Taken together, these studies suggest a model in which NK cells shift the balance of lung inflammation away from a pro-fibrotic response, perhaps via cytokine production and protection against infection, and these functions of NK cells are impaired in smokers and patients with fibrotic lung disease.^{165,166,174} Thus, enhancement of NK cell function may offer novel therapeutic approaches to these debilitating and often fatal diseases.

Finally, although NK cell activation may be beneficial in reducing lung fibrosis, NK cells may contribute to loss of lung parenchymal cells in COPD. MICA is expressed on the airway epithelium of COPD patients, and expression of the murine NKG2D ligand Rae-1 on lung epithelium leads to emphysema-like pulmonary dysfunction in mice, which is blocked by treatment with anti-NKG2D or NK cell depletion.¹⁷⁵

Conclusions and future questions

NK cells may tip the balance between health and pathology in the lung, and thus understanding their actions may identify novel targets for immunomodulation in respiratory disease. NK cells are activated by multiple mechanisms in the lung and protect against viral, bacterial and fungal infection, through direct antiviral actions and activation of macrophages, dendritic cells and the adaptive immune response. NK cells are also activated in chronic inflammatory diseases of the lung. Although their role in these diseases is not fully understood, their ability to produce 'Th2' cytokines may promote lung inflammation, whereas their production of IFN- γ , and other actions, may reduce lung fibrosis. The activation status of

NK cells may have dual implications for chronic inflammatory diseases, such as asthma and COPD, which are exacerbated by respiratory infection.

Many important gaps remain in our understanding of the NK cell response in the lung. NK cells can be deficient or altered in phenotype in respiratory diseases, but whether this is a reflection of the ongoing pathological process or a cause of increased susceptibility to disease is often unclear. Although many potential interactions of NK cells with dendritic cells, macrophages and T cells have been demonstrated *in vitro*, their location, timing and importance during different phases of an ongoing respiratory infection or inflammatory response are still largely unknown, as is the role of different NK subsets. The lung has unique properties which regulate immune responses and, as NK cells specialize their function in peripheral tissues, it will be interesting to discover whether NK cells also specialize their phenotype to the pulmonary environment in homeostasis and disease. It will also be important to know whether 'memory' or long-term changes in NK cell responses can result from or determine respiratory health. Finally, how the NK cell response is down-regulated after a pathogen has been cleared or to prevent pathology during inflammation, is another area that could provide insights into the mechanisms underlying important respiratory diseases.

Acknowledgements

I am grateful to Daniel M. Davis, Philipp Eissmann, Suzie M. Hingley-Wilson and Salim I. Khakoo for critical reading of this manuscript.

Disclosures

The author has no conflicts of interest to disclose.

References

- 1 Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol* 2008; **9**:503–10.
- 2 Lodoen MB, Lanier LL. Natural killer cells as an initial defense against pathogens. *Curr Opin Immunol* 2006; **18**:391–8.
- 3 Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural-killer cells and dendritic cells: "l'union fait la force". *Blood* 2005; **106**:2252–8.
- 4 Nedvetzki S, Sowinski S, Eagle RA *et al*. Reciprocal regulation of human natural killer cells and macrophages associated with distinct immune synapses. *Blood* 2007; **109**:3776–85.
- 5 Davis DM, Chiu I, Fassett M, Cohen GB, Mandelboim O, Strominger JL. The human natural killer cell immune synapse. *Proc Natl Acad Sci USA* 1999; **96**:15062–7.
- 6 Davis DM, Dustin ML. What is the importance of the immunological synapse? *Trends Immunol* 2004; **25**:323–7.
- 7 Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol* 2008; **9**:495–502.

- 8 Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature* 2005; **436**:1186–90.
- 9 Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 1999; **285**:727–9.
- 10 Braud VM, Allan DS, O’Callaghan CA *et al.* HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 1998; **391**:795–9.
- 11 Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 1986; **319**:675–8.
- 12 Biron CA. Interferons [alpha] and [beta] as immune regulators – a new look. *Immunity* 2001; **14**:661–4.
- 13 Biron C. Initial and innate responses to viral infections – pattern setting in immunity or disease. *Curr Opin Microbiol* 1999; **2**:374–81.
- 14 Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol* 1999; **17**:189–220.
- 15 Orange JS. Formation and function of the lytic NK-cell immunological synapse. *Nat Rev Immunol* 2008; **8**:713–25.
- 16 Maroof A, Beattie L, Zubairi S, Svensson M, Stager S, Kaye PM. Posttranscriptional regulation of il10 gene expression allows natural killer cells to express immunoregulatory function. *Immunity* 2008; **29**:295–305.
- 17 Di Santo JP. Natural killer cells: diversity in search of a niche. *Nat Immunol* 2008; **9**:473–5.
- 18 Colonna M. Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity. *Immunity* 2009; **31**:15–23.
- 19 Vivier E, Spits H, Cupedo T. Interleukin-22-producing innate immune cells: new players in mucosal immunity and tissue repair? *Nat Rev Immunol* 2009; **9**:229–34.
- 20 Di Santo JP. Functionally distinct NK-cell subsets: developmental origins and biological implications. *Eur J Immunol* 2008; **38**:2948–51.
- 21 Moretta A. Natural killer cells and dendritic cells: rendezvous in abused tissues. *Nat Rev Immunol* 2002; **2**:957–64.
- 22 Andoniou CE, Coudert JD, Degli-Esposti MA. Killers and beyond: NK-cell-mediated control of immune responses. *Eur J Immunol* 2008; **38**:2938–42.
- 23 Martin-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, Sallusto F. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. *Nat Immunol* 2004; **5**:1260–5.
- 24 Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature* 2009; **457**:557–61.
- 25 O’Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol* 2006; **7**:507–16.
- 26 Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci USA* 2009; **106**:1915–9.
- 27 Gregoire C, Chasson L, Luci C, Tomasello E, Geissmann F, Vivier E, Walzer T. The trafficking of natural killer cells. *Immunol Rev* 2007; **220**:169–82.
- 28 Stein-Streilein J, Bennett M, Mann D, Kumar V. Natural killer cells in mouse lung: surface phenotype, target preference, and response to local influenza virus infection. *J Immunol* 1983; **131**:2699–704.
- 29 Reynolds CW, Timonen T, Herberman RB. Natural killer (NK) cell activity in the rat. I. Isolation and characterization of the effector cells. *J Immunol* 1981; **127**:282–7.
- 30 Ge N, Nishioka Y, Nakamura Y *et al.* Synthesis and secretion of interleukin-15 by freshly isolated human bronchial epithelial cells. *Int Arch Allergy Immunol* 2004; **135**:235–42.
- 31 Leung KN, Ada GL. Induction of natural killer cells during murine influenza virus infection. *Immunobiology* 1981; **160**:352–66.
- 32 Schuster M, Tschernig T, Krug N, Pabst R. Lymphocytes migrate from the blood into the bronchoalveolar lavage and lung parenchyma in the asthma model of the brown Norway rat. *Am J Respir Crit Care Med* 2000; **161** (2 Pt 1):558–66.
- 33 Morrison BE, Park SJ, Mooney JM, Mehrad B. Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis. *J Clin Invest* 2003; **112**:1862–70.
- 34 Kim S, Iizuka K, Kang HS, Dokun A, French AR, Greco S, Yokoyama WM. In vivo developmental stages in murine natural killer cell maturation. *Nat Immunol* 2002; **3**:523–8.
- 35 Stein-Streilein J, Guffee J, Fan W. Locally and systemically derived natural killer cells participate in defense against intranasally inoculated influenza virus. *Reg Immunol* 1988; **1**:100–5.
- 36 Wissinger E, Goulding J, Hussell T. Immune homeostasis in the respiratory tract and its impact on heterologous infection. *Semin Immunol* 2009; **21**:147–55.
- 37 Bordignon C, Villa F, Vecchi A, Giavazzi R, Introna M, Avallone R, Mantovani A. Natural cytotoxic activity in human lungs. *Clin Exp Immunol* 1982; **47**:437–44.
- 38 Robinson BW, Pinkston P, Crystal RG. Natural killer cells are present in the normal human lung but are functionally incompetent. *J Clin Invest* 1984; **74**:942–50.
- 39 Weissman DN, deShazo RD, Banks DE. Modulation of natural killer cell function by human alveolar macrophages. *J Allergy Clin Immunol* 1986; **78** (4 Pt 1):571–7.
- 40 Roth MD, Golub SH. Inhibition of lymphokine-activated killer cell function by human alveolar macrophages. *Cancer Res* 1989; **49**:4690–5.
- 41 Bordignon C, Villa F, Allavena P, Introna M, Biondi A, Avallone R, Mantovani A. Inhibition of natural killer activity by human bronchoalveolar macrophages. *J Immunol* 1982; **129**:587–91.
- 42 Laouar Y, Sutterwala FS, Gorelik L, Flavell RA. Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. *Nat Immunol* 2005; **6**:600–7.
- 43 Lauzon W, Lemaire I. Alveolar macrophage inhibition of lung-associated NK activity: involvement of prostaglandins and transforming growth factor-beta 1. *Exp Lung Res* 1994; **20**:331–49.
- 44 Wilsher ML, Hughes DA, Haslam PL. Immunomodulatory effects of pulmonary surfactant on natural killer cell and antibody-dependent cytotoxicity. *Clin Exp Immunol* 1988; **74**:465–70.
- 45 Pangault C, Le Fric G, Caulet-Maugendre S, Lena H, Amiot L, Guilloux V, Onno M, Fauchet R. Lung macrophages and dendritic cells express HLA-G molecules in pulmonary diseases. *Hum Immunol* 2002; **63**:83–90.
- 46 Okamoto M, Kato S, Oizumi K *et al.* Interleukin 18 (IL-18) in synergy with IL-2 induces lethal lung injury in mice: a potential role for cytokines, chemokines, and natural killer cells in the pathogenesis of interstitial pneumonia. *Blood* 2002; **99**:1289–98.
- 47 Zimmer J, Donato L, Hanau D, Cazenave J-P, Tongio M-M, Moretta A, de la Salle H. Activity and Phenotype of Natural

- Killer Cells in Peptide Transporter (TAP)-deficient Patients (Type I Bare Lymphocyte Syndrome). *J Exp Med* 1998; **187**:117–22.
- 48 Hanna J, Mussaffi H, Steuer G *et al*. Functional aberrant expression of CCR2 receptor on chronically activated NK cells in patients with TAP-2 deficiency. *Blood* 2005; **106**:3465–73.
 - 49 Moins-Teisserenc HT, Gadola SD, Cella M *et al*. Association of a syndrome resembling Wegener's granulomatosis with low surface expression of HLA class-I molecules. *The Lancet* 1999; **354**:1598–603.
 - 50 Komiyama A, Kawai H, Yamada S, Kato M, Yanagisawa M, Miyagawa Y, Akabane T. A killing defect of natural killer cells with the absence of natural killer cytotoxic factors in a child with Hodgkin's disease. *Blood* 1987; **69**:1686–90.
 - 51 Komiyama A, Kawai H, Yabuhara A, Yanagisawa M, Miyagawa Y, Ota M, Hasekura H, Akabane T. Natural killer cell immunodeficiency in siblings: defective killing in the absence of natural killer cytotoxic factor activity in natural killer and lymphokine-activated killer cytotoxicities. *Pediatrics* 1990; **85**:323–30.
 - 52 Orange JS. Human natural killer cell deficiencies. *Curr Opin Allergy Clin Immunol* 2006; **6**:399–409.
 - 53 Orange JS. Human natural killer cell deficiencies and susceptibility to infection. *Microbes Infect* 2002; **4**:1545–58.
 - 54 Jawahar S, Moody C, Chan M, Finberg R, Geha R, Chatila T. Natural Killer (NK) cell deficiency associated with an epitope-deficient Fc receptor type IIIA (CD16-II). *Clin Exp Immunol* 1996; **103**:408–13.
 - 55 Eidenschenk C, Dunne J, Jouanguy E *et al*. A novel primary immunodeficiency with specific natural-killer cell deficiency maps to the centromeric region of chromosome 8. *Am J Hum Genet* 2006; **78**:721–7.
 - 56 Salomon R, Webster RG. The influenza virus enigma. *Cell* 2009; **136**:402–10.
 - 57 Hikono H, Kohlmeier JE, Ely KH, Scott I, Roberts AD, Blackman MA, Woodland DL. T-cell memory and recall responses to respiratory virus infections. *Immunol Rev* 2006; **211**:119–32.
 - 58 Doherty PC, Turner SJ, Webby RG, Thomas PG. Influenza and the challenge for immunology. *Nat Immunol* 2006; **7**:449–55.
 - 59 Welliver TP, Garofalo RP, Hosakote Y *et al*. Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses. *J Infect Dis* 2007; **195**:1126–36.
 - 60 Welliver TP, Reed JL, Welliver RC Sr. Respiratory syncytial virus and influenza virus infections: observations from tissues of fatal infant cases. *Pediatr Infect Dis J* 2008; **27**(Suppl. 10):S92–6.
 - 61 Hayden FG, Fritz R, Lobo MC, Alvord W, Strober W, Straus SE. Local and systemic cytokine responses during experimental human influenza a virus infection. Relation to symptom formation and host defense. *J Clin Invest* 1998; **101**:643–9.
 - 62 Kash JC, Tumpey TM, Prohl SC *et al*. Genomic analysis of increased host immune and cell death responses induced by 1918 influenza virus. *Nature* 2006; **443**:578–81.
 - 63 de Jong MD, Simmons CP, Thanh TT *et al*. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006; **12**:1203–7.
 - 64 Kobasa D, Jones SM, Shinya K *et al*. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature* 2007; **445**:319–23.
 - 65 Loo YM, Gale M Jr. Influenza: fatal immunity and the 1918 virus. *Nature* 2007; **445**:267–8.
 - 66 Ennis FA, Meager A, Beare AS, Qi YH, Riley D, Schwarz G, Schild GC, Rook AH. Interferon induction and increased natural killer-cell activity in influenza infections in man. *Lancet* 1981; **2**:891–3.
 - 67 Nogusa S, Ritz BW, Kassim SH, Jennings SR, Gardner EM. Characterization of age-related changes in natural killer cells during primary influenza infection in mice. *Mech Ageing Dev* 2008; **129**:223–30.
 - 68 Stein-Streilein J, Guffee J. In vivo treatment of mice and hamsters with antibodies to asialo GM1 increases morbidity and mortality to pulmonary influenza infection. *J Immunol* 1986; **136**:1435–41.
 - 69 Kos FJ, Engleman EG. Role of natural killer cells in the generation of Influenza virus-specific cytotoxic T cells. *Cell Immunol* 1996; **173**:1–6.
 - 70 He XS, Draghi M, Mahmood K *et al*. T cell-dependent production of IFN-gamma by NK cells in response to influenza A virus. *J Clin Invest* 2004; **114**:1812–9.
 - 71 Gazit R, Gruda R, Elboim M *et al*. Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. *Nat Immunol* 2006; **7**:517–23.
 - 72 Arnon TI, Lev M, Katz G, Chernobrov Y, Porgador A, Mandelboim O. Recognition of viral hemagglutinins by NKp44 but not by NKp30. *Eur J Immunol* 2001; **31**:2680–9.
 - 73 Arnon TI, Achdout H, Lieberman N *et al*. The mechanisms controlling the recognition of tumor- and virus-infected cells by NKp46. *Blood* 2004; **103**:664–72.
 - 74 Mandelboim O, Lieberman N, Lev M *et al*. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* 2001; **409**:1055–60.
 - 75 Ho JW, Hershkovitz O, Peiris M *et al*. H5-type influenza virus hemagglutinin is functionally recognized by the natural killer-activating receptor NKp44. *J Virol* 2008; **82**:2028–32.
 - 76 Owen RE, Yamada E, Thompson CI *et al*. Alterations in receptor binding properties of recent human influenza H3N2 viruses are associated with reduced natural killer cell lysis of infected cells. *J Virol* 2007; **81**:11170–8.
 - 77 Siren J, Sareneva T, Pirhonen J, Strengell M, Veckman V, Julkunen I, Matikainen S. Cytokine and contact-dependent activation of natural killer cells by influenza A or Sendai virus-infected macrophages. *J Gen Virol* 2004; **85** (Pt 8):2357–64.
 - 78 Draghi M, Pashine A, Sanjanwala B *et al*. NKp46 and NKG2D recognition of infected dendritic cells is necessary for NK cell activation in the human response to influenza infection. *J Immunol* 2007; **178**:2688–98.
 - 79 Ebihara T, Masuda H, Akazawa T, Shingai M, Kikuta H, Ariga T, Matsumoto M, Seya T. Induction of NKG2D ligands on human dendritic cells by TLR ligand stimulation and RNA virus infection. *Int Immunol* 2007; **19**:1145–55.
 - 80 Achdout H, Arnon TI, Markel G *et al*. Enhanced recognition of human NK receptors after influenza virus infection. *J Immunol* 2003; **171**:915–23.
 - 81 Achdout H, Manaster I, Mandelboim O. Influenza virus infection augments NK cell inhibition through reorganization of major histocompatibility complex class I proteins. *J Virol* 2008; **82**:8030–7.
 - 82 Ahlenstiel G, Martin MP, Gao X, Carrington M, Rehermann B. Distinct KIR/HLA compound genotypes affect the kinetics of

- human antiviral natural killer cell responses. *J Clin Invest* 2008; **118**:1017–26.
- 83 Jegerlehner A, Schmitz N, Storni T, Bachmann MF. Influenza A vaccine based on the extracellular domain of M2: weak protection mediated via antibody-dependent NK cell activity. *J Immunol* 2004; **172**:5598–605.
- 84 Ishikawa E, Nakazawa M, Yoshinari M, Minami M. Role of tumor necrosis factor-related apoptosis-inducing ligand in immune response to influenza virus infection in mice. *J Virol* 2005; **79**:7658–63.
- 85 Liu B, Mori I, Hossain MJ, Dong L, Takeda K, Kimura Y. Interleukin-18 improves the early defence system against influenza virus infection by augmenting natural killer cell-mediated cytotoxicity. *J Gen Virol* 2004; **85** (Pt 2):423–8.
- 86 Van Der Sluijs KF, Van Elden LJ, Arens R *et al.* Enhanced viral clearance in interleukin-18 gene-deficient mice after pulmonary infection with influenza A virus. *Immunology* 2005; **114**:112–20.
- 87 WHO. *Global Tuberculosis Control: Epidemiology, Strategy, Financing: WHO report 2009*. Geneva, Switzerland: WHO Press, World Health Organisation, 2009. ISBN 978 92 4 156380 2.
- 88 Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J Exp Med* 1993; **178**:2243–7.
- 89 Al-Muhsen S, Casanova JL. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. *J Allergy Clin Immunol* 2008; **122**:1043–51; quiz 52–3.
- 90 Korbel DS, Schneider BE, Schaible UE. Innate immunity in tuberculosis: myths and truth. *Microbes Infect* 2008; **10**:995–1004.
- 91 Millman AC, Salman M, Dayaram YK, Connell ND, Venketaraman V. Natural killer cells, glutathione, cytokines, and innate immunity against *Mycobacterium tuberculosis*. *J Interferon Cytokine Res* 2008; **28**:153–65.
- 92 Schierloh P, Aleman M, Yokobori N, Alves L, Roldan N, Abbate E, del CSM, de la Barrera S. NK cell activity in tuberculosis is associated with impaired CD11a and ICAM-1 expression: a regulatory role of monocytes in NK activation. *Immunology* 2005; **116**:541–52.
- 93 Vankayalapati R, Wizel B, Weis SE *et al.* The Nkp46 receptor contributes to NK cell lysis of mononuclear phagocytes infected with an intracellular bacterium. *J Immunol* 2002; **168**:3451–7.
- 94 Schierloh P, Yokobori N, Aleman M *et al.* Increased susceptibility to apoptosis of CD56dim CD16 + NK cells induces the enrichment of IFN-gamma-producing CD56bright cells in tuberculous pleurisy. *J Immunol* 2005; **175**:6852–60.
- 95 Denis M. Interleukin-12 (IL-12) augments cytolytic activity of natural killer cells toward *Mycobacterium tuberculosis*-infected human monocytes. *Cell Immunol* 1994; **156**:529–36.
- 96 Brill KJ, Li Q, Larkin R, Canaday DH, Kaplan DR, Boom WH, Silver RF. Human natural killer cells mediate killing of intracellular *Mycobacterium tuberculosis* H37Rv via granule-independent mechanisms. *Infect Immun* 2001; **69**:1755–65.
- 97 Vankayalapati R, Garg A, Porgador A *et al.* Role of NK cell-activating receptors and their ligands in the lysis of mononuclear phagocytes infected with an intracellular bacterium. *J Immunol* 2005; **175**:4611–7.
- 98 Garg A, Barnes PF, Porgador A *et al.* Vimentin expressed on *Mycobacterium tuberculosis*-infected human monocytes is involved in binding to the Nkp46 receptor. *J Immunol* 2006; **177**:6192–8.
- 99 Esin S, Batoni G, Pardini M *et al.* Functional characterization of human natural killer cells responding to *Mycobacterium bovis* bacille Calmette-Guerin. *Immunology* 2004; **112**:143–52.
- 100 Esin S, Batoni G, Counoupas C *et al.* Direct binding of human NK cell natural cytotoxicity receptor NKp44 to the surfaces of mycobacteria and other bacteria. *Infect Immun* 2008; **76**:1719–27.
- 101 Shojima J, Tanaka G, Keicho N *et al.* Identification of MICA as a susceptibility gene for pulmonary *Mycobacterium avium* complex infection. *J Infect Dis* 2009; **30**:30.
- 102 Vankayalapati R, Klucar P, Wizel B, Weis SE, Samten B, Safi H, Shams H, Barnes PF. NK cells regulate CD8+ T cell effector function in response to an intracellular pathogen. *J Immunol* 2004; **172**:130–7.
- 103 Roy S, Barnes PF, Garg A, Wu S, Cosman D, Vankayalapati R. NK cells lyse T regulatory cells that expand in response to an intracellular pathogen. *J Immunol* 2008; **180**:1729–36.
- 104 Junqueira-Kipnis AP, Kipnis A, Jamieson A, Juarrero MG, Diefenbach A, Raulet DH, Turner J, Orme IM. NK cells respond to pulmonary infection with *Mycobacterium tuberculosis*, but play a minimal role in protection. *J Immunol* 2003; **171**:6039–45.
- 105 Woolard MD, Hudig D, Tabor L, Ivey JA, Simecka JW. NK Cells in Gamma-Interferon-Deficient Mice Suppress Lung Innate Immunity against *Mycoplasma* spp. *Infect Immun* 2005; **73**:6742–51.
- 106 Saxena RK, Weissman D, Saxena QB, Simpson J, Lewis DM. Kinetics of changes in lymphocyte sub-populations in mouse lungs after intrapulmonary infection with *M. bovis* (Bacillus Calmette-Guerin) and identity of cells responsible for IFN-gamma responses. *Clin Exp Immunol* 2002; **128**:405–10.
- 107 Saxena RK, Weissman D, Simpson J, Lewis DM. Murine model of BCG lung infection: dynamics of lymphocyte subpopulations in lung interstitium and tracheal lymph nodes. *J Biosci* 2002; **27**:143–53.
- 108 Feng CG, Kaviratne M, Rothfuchs AG, Cheever A, Hieny S, Young HA, Wynn TA, Sher A. NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with *Mycobacterium tuberculosis*. *J Immunol* 2006; **177**:7086–93.
- 109 Orme IM. The mouse as a useful model of tuberculosis. *Tuberculosis* 2003; **83**:112–5.
- 110 WHO. *Global Surveillance, Prevention and Control of Chronic Respiratory Diseases: a Comprehensive Approach*. Geneva, Switzerland: WHO Press, World Health Organisation, 2007. ISBN 978 92 4 156346 8.
- 111 Lloyd CM, Robinson DS. Allergen-induced airway remodelling. *Eur Respir J* 2007; **29**:1020–32.
- 112 Jira M, Antosova E, Vondra V, Strejcek J, Mazakova H, Prazakova J. Natural killer and interleukin-2 induced cytotoxicity in asthmatics. I. Effect of acute antigen-specific challenge. *Allergy* 1988; **43**:294–8.
- 113 Timonen T, Stenius-Aarniala B. Natural killer cell activity in asthma. *Clin Exp Immunol* 1985; **59**:85–90.
- 114 Di Lorenzo G, Esposito Pellitteri M, Drago A, Di Blasi P, Candore G, Balistreri C, Listi F, Caruso C. Effects of in vitro treatment with fluticasone propionate on natural killer and lymphokine-induced killer activity in asthmatic and healthy individuals. *Allergy* 2001; **56**:323–7.
- 115 Burke SM, Issekutz TB, Mohan K, Lee PW, Shmulevitz M, Marshall JS. Human mast cell activation with virus-associated

- stimuli leads to the selective chemotaxis of natural killer cells by a CXCL8-dependent mechanism. *Blood* 2008; **111**:5467–76.
- 116 Lin SJ, Chang LY, Yan DC, Huang YJ, Lin TJ, Lin TY. Decreased intercellular adhesion molecule-1 (CD54) and L-selectin (CD62L) expression on peripheral blood natural killer cells in asthmatic children with acute exacerbation. *Allergy* 2003; **58**:67–71.
 - 117 Wei H, Zhang J, Xiao W, Feng J, Sun R, Tian Z. Involvement of human natural killer cells in asthma pathogenesis: natural killer 2 cells in type 2 cytokine predominance. *J Allergy Clin Immunol* 2005; **115**:841–7.
 - 118 Ozdemir O. Type 2 natural killer cells in asthma? *J Allergy Clin Immunol* 2005; **116**:1165–6; author reply 6–7.
 - 119 Aktas E, Akdis M, Bilgic S, Disch R, Falk CS, Blaser K, Akdis C, Deniz G. Different natural killer (NK) receptor expression and immunoglobulin E (IgE) regulation by NK1 and NK2 cells. *Clin Exp Immunol* 2005; **140**:301–9.
 - 120 Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaehri BA, Ghayur T, Carson WE, Caligiuri MA. Human natural killer cells: a unique innate immunoregulatory role for the CD56bright subset. *Blood* 2001; **97**:3146–51.
 - 121 Babu S, Blauvelt CP, Nutman TB. Filarial parasites induce NK cell activation, type 1 and type 2 cytokine secretion, and subsequent apoptotic cell death. *J Immunol* 2007; **179**:2445–56.
 - 122 Deniz G, Akdis M, Aktas E, Blaser K, Akdis CA. Human NK1 and NK2 subsets determined by purification of IFN-gamma-secreting and IFN-gamma-nonsecreting NK cells. *Eur J Immunol* 2002; **32**:879–84.
 - 123 Warren HS, Kinnear BF, Phillips JH, Lanier LL. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. *J Immunol* 1995; **154**:5144–52.
 - 124 Peritt D, Robertson S, Gri G, Showe L, Aste-Amezaga M, Trinchieri G. Cutting edge: differentiation of human NK cells into NK1 and NK2 subsets. *J Immunol* 1998; **161**:5821–4.
 - 125 Warren HS, Kinnear BF, Kastelein RL, Lanier LL. Analysis of the costimulatory role of IL-2 and IL-15 in initiating proliferation of resting (CD56dim) human NK cells. *J Immunol* 1996; **156**:3254–9.
 - 126 Hoshino T, Winkler-Pickett RT, Mason AT, Ortaldo JR, Young HA. IL-13 Production by NK Cells: IL-13-Producing NK and T Cells Are Present In Vivo in the Absence of IFN- γ . *J Immunol* 1999; **162**:51–9.
 - 127 Hoshino T, Wiltout RH, Young HA. IL-18 is a potent coinducer of IL-13 in NK and T Cells: a new potential role for IL-18 in modulating the immune response. *J Immunol* 1999; **162**:5070–7.
 - 128 Thornton S, Kuhn KA, Finkelman FD, Hirsch R. NK cells secrete high levels of IFN-gamma in response to in vivo administration of IL-2. *Eur J Immunol* 2001; **31**:3355–60.
 - 129 Fehniger TA, Shah MH, Turner MJ *et al.* Differential cytokine and chemokine gene expression by human NK Cells following activation with IL-18 or IL-15 in combination with IL-12: implications for the innate immune response. *J Immunol* 1999; **162**:4511–20.
 - 130 Loza MJ, Perussia B. Final steps of natural killer cell maturation: a model for type 1-type 2 differentiation? *Nat Immunol* 2001; **2**:917–24.
 - 131 Loza MJ, Zamai L, Azzoni L, Rosati E, Perussia B. Expression of type 1 (interferon gamma) and type 2 (interleukin-13, interleukin-5) cytokines at distinct stages of natural killer cell differentiation from progenitor cells. *Blood* 2002; **99**:1273–81.
 - 132 Loza MJ, Peters SP, Zangrilli JG, Perussia B. Distinction between IL-13+ and IFN-gamma+ natural killer cells and regulation of their pool size by IL-4. *Eur J Immunol* 2002; **32**:413–23.
 - 133 Colonna M. Can we apply the TH1-TH2 paradigm to all lymphocytes? *Nat Immunol* 2001; **2**:899–900.
 - 134 Perussia B, Loza MJ. Linear “2-0-1” lymphocyte development: hypotheses on cellular bases for immunity. *Trends Immunol* 2003; **24**:235–41.
 - 135 Hussell T, Openshaw PJ. Intracellular IFN-gamma expression in natural killer cells precedes lung CD8+ T cell recruitment during respiratory syncytial virus infection. *J Gen Virol* 1998; **79**:2593–601.
 - 136 McDermott JR, Humphreys NE, Forman SP, Donaldson DD, Grecis RK. Intraepithelial NK cell-derived IL-13 induces intestinal pathology associated with nematode infection. *J Immunol* 2005; **175**:3207–13.
 - 137 Morris SC, Orekhova T, Meadows MJ, Heidorn SM, Yang J, Finkelman FD. IL-4 induces in vivo production of IFN-gamma by NK and NKT cells. *J Immunol* 2006; **176**:5299–305.
 - 138 Pettipher R, Hansel TT, Armer R. Antagonism of the prostaglandin D2 receptors DP1 and CRTH2 as an approach to treat allergic diseases. *Nat Rev Drug Discov* 2007; **6**:313–25.
 - 139 Chen Y, Perussia B, Campbell KS. Prostaglandin D2 suppresses human NK cell function via signaling through D prostanoid receptor. *J Immunol* 2007; **179**:2766–73.
 - 140 Contoli M, Message SD, Laza-Stanca V *et al.* Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med* 2006; **12**:1023–6.
 - 141 Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; **201**:937–47.
 - 142 Marcenaro E, Chiesa MD, Bellora F, Parolini S, Millo R, Moretta L, Moretta A. IL-12 or IL-4 Prime Human NK Cells to Mediate Functionally Divergent Interactions with Dendritic Cells or Tumors. *J Immunol* 2005; **174**:3992–8.
 - 143 Scordamaglia F, Balsamo M, Scordamaglia A, Moretta A, Mingari MC, Canonica GW, Moretta L, Vitale M. Perturbations of natural killer cell regulatory functions in respiratory allergic diseases. *J Allergy Clin Immunol* 2008; **121**:479–85.
 - 144 Wingett D, Nielson CP. Divergence in NK cell and cyclic AMP regulation of T cell CD40L expression in asthmatic subjects. *J Leukoc Biol* 2003; **74**:531–41.
 - 145 Message SD, Laza-Stanca V, Mallia P *et al.* Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci USA* 2008; **105**:13562–7.
 - 146 Sykes A, Johnston SL. Etiology of asthma exacerbations. *J Allergy Clin Immunol* 2008; **122**:685–8.
 - 147 Korsgren M, Persson CG, Sundler F *et al.* Natural killer cells determine development of allergen-induced eosinophilic airway inflammation in mice. *J Exp Med* 1999; **189**:553–62.
 - 148 Scharton TM, Scott P. Natural killer cells are a source of interferon gamma that drives differentiation of CD4+ T cell subsets and induces early resistance to *Leishmania major* in mice. *J Exp Med* 1993; **178**:567–77.
 - 149 Han X, Fan Y, Wang S, Jiao L, Qiu H, Yang X. NK cells contribute to intracellular bacterial infection-mediated inhibition of allergic responses. *J Immunol* 2008; **180**:4621–8.

- 150 Hussell T, Openshaw PJ. IL-12-activated NK cells reduce lung eosinophilia to the attachment protein of respiratory syncytial virus but do not enhance the severity of illness in CD8 T cell-immunodeficient conditions. *J Immunol* 2000; **165**:7109–15.
- 151 Walker C, Checkel J, Cammisuli S, Leibson PJ, Gleich GJ. IL-5 Production by NK Cells Contributes to Eosinophil Infiltration in a Mouse Model of Allergic Inflammation. *J Immunol* 1998; **161**:1962–9.
- 152 Matsubara S, Takeda K, Kodama T *et al.* IL-2 and IL-18 attenuation of airway hyperresponsiveness requires STAT4, IFN-gamma, and natural killer cells. *Am J Respir Cell Mol Biol* 2007; **36**:324–32.
- 153 Wilson MS, Wynn TA. Pulmonary fibrosis: pathogenesis, etiology and regulation. *Mucosal Immunol* 2009; **2**:103–21.
- 154 Cosio MG, Sassetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. *N Engl J Med* 2009; **360**:2445–54.
- 155 Takeuchi M, Nagai S, Nakajima A *et al.* Inhibition of lung natural killer cell activity by smoking: the role of alveolar macrophages. *Respiration* 2001; **68**:262–7.
- 156 Ferson M, Edwards A, Lind A, Milton GW, Hersey P. Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. *Int J Cancer* 1979; **23**:603–9.
- 157 Zeidel A. Immune response in asymptomatic smokers. *Acta Anaesthesiol Scand* 2002; **46**:959–64.
- 158 Lu LM, Zavitz CC, Chen B, Kianpour S, Wan Y, Stampfli MR. Cigarette smoke impairs NK cell-dependent tumor immune surveillance. *J Immunol* 2007; **178**:936–43.
- 159 Prieto A, Reyes E, Bernstein ED *et al.* Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycoprophosphopeptical (immunoferrin). *Am J Respir Crit Care Med* 2001; **163**:1578–83.
- 160 Fairclough L, Urbanowicz RA, Corne J, Lamb JR. Killer cells in chronic obstructive pulmonary disease. *Clin Sci (Lond)* 2008; **114**:533–41.
- 161 Khalil N, Parekh TV, O'Connor R, Antman N, Kepron W, Yehaulaeshet T, Xu YD, Gold LI. Regulation of the effects of TGF-beta 1 by activation of latent TGF-beta 1 and differential expression of TGF-beta receptors (T beta R-I and T beta R-II) in idiopathic pulmonary fibrosis. *Thorax* 2001; **56**:907–15.
- 162 Lee JC, Lee KM, Kim DW, Heo DS. Elevated TGF-beta1 secretion and down-modulation of NKG2D underlies impaired NK cytotoxicity in cancer patients. *J Immunol* 2004; **172**:7335–40.
- 163 Aquino-Galvez A, Perez-Rodriguez M, Camarena A *et al.* MICA polymorphisms and decreased expression of the MICA receptor NKG2D contribute to idiopathic pulmonary fibrosis susceptibility. *Hum Genet* 2009; **12**:12.
- 164 Jiang D, Liang J, Hodge J *et al.* Regulation of pulmonary fibrosis by chemokine receptor CXCR3. *J Clin Invest* 2004; **114**:291–9.
- 165 Strieter RM, Keane MP. Innate immunity dictates cytokine polarization relevant to the development of pulmonary fibrosis. *J Clin Invest* 2004; **114**:165–8.
- 166 Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA, King TE Jr. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2004; **350**:125–33.
- 167 King TE Jr, Albera C, Bradford WZ *et al.* Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. *The Lancet* 2009; **374**:222–8.
- 168 Krizhanovsky V, Yon M, Dickins RA *et al.* Senescence of activated stellate cells limits liver fibrosis. *Cell* 2008; **134**:657–67.
- 169 Radaeva S, Sun R, Jaruga B, Nguyen VT, Tian Z, Gao B. Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners. *Gastroenterology* 2006; **130**:435–52.
- 170 Melhem A, Muhanna N, Bishara A *et al.* Anti-fibrotic activity of NK cells in experimental liver injury through killing of activated HSC. *J Hepatol* 2006; **45**:60–71.
- 171 Notas G, Kisseleva T, Brenner D. NK and NKT cells in liver injury and fibrosis. *Clin Immunol* 2009; **130**:16–26.
- 172 Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. *N Engl J Med* 2008; **359**:2355–65.
- 173 Wesselkamper SC, Eppert BL, Motz GT, Lau GW, Hassett DJ, Borchers MT. NKG2D is critical for NK cell activation in host defense against *Pseudomonas aeruginosa* respiratory infection. *J Immunol* 2008; **181**:5481–9.
- 174 Coker RK, Laurent GJ. Pulmonary fibrosis: cytokines in the balance. *Eur Respir J* 1998; **11**:1218–21.
- 175 Borchers MT, Wesselkamper SC, Curull V *et al.* Sustained CTL activation by murine pulmonary epithelial cells promotes the development of COPD-like disease. *J Clin Invest* 2009; **119**:636–49.
- 176 Kawakami K, Koguchi Y, Qureshi MH *et al.* IL-18 contributes to host resistance against infection with *Cryptococcus neoformans* in mice with defective IL-12 synthesis through induction of IFN-gamma production by NK cells. *J Immunol* 2000; **165**:941–7.
- 177 Park SJ, Hughes MA, Burdick M, Strieter RM, Mehrad B. Early NK cell-derived IFN- γ is essential to host defense in neutropenic invasive aspergillosis. *J Immunol* 2009; **182**:4306–12.
- 178 Byrne P, McGuirk P, Todryk S, Mills KH. Depletion of NK cells results in disseminating lethal infection with *Bordetella pertussis* associated with a reduction of antigen-specific Th1 and enhancement of Th2, but not Tr1 cells. *Eur J Immunol* 2004; **34**:2579–88.
- 179 Kerr AR, Kirkham LA, Kadioglu A, Andrew PW, Garside P, Thompson H, Mitchell TJ. Identification of a detrimental role for NK cells in pneumococcal pneumonia and sepsis in immunocompromised hosts. *Microbes Infect* 2005; **7**:845–52.
- 180 Lopez MC, Duckett NS, Baron SD, Metzger DW. Early activation of NK cells after lung infection with the intracellular bacterium, *Francisella tularensis* LVS. *Cell Immunol* 2004; **232**:75–85.
- 181 Sporri R, Joller N, Albers U, Hilbi H, Oxenius A. MyD88-dependent IFN-gamma production by NK cells is key for control of *Legionella pneumophila* infection. *J Immunol* 2006; **176**:6162–71.
- 182 Miyazaki S, Ishikawa F, Shimizu K, Ubagai T, Edelstein PH, Yamaguchi K. Gr-1-high polymorphonuclear leukocytes and NK cells act via IL-15 to clear intracellular *Haemophilus influenzae* in experimental murine peritonitis and pneumonia. *J Immunol* 2007; **179**:5407–14.
- 183 Borchers MT, Harris NL, Wesselkamper SC, Zhang S, Chen Y, Young L, Lau GW. The NKG2D-Activating Receptor Mediates Pulmonary Clearance of *Pseudomonas aeruginosa*. *Infect Immun* 2006; **74**:2578–86.
- 184 D'Orazio JA, Burke GW, Stein-Streilein J. Staphylococcal enterotoxin B activates purified NK cells to secrete IFN-gamma but requires T lymphocytes to augment NK cytotoxicity. *J Immunol* 1995; **154**:1014–23.

- 185 Yoshihara R, Shiozawa S, Fujita T, Chihara K. Gamma interferon is produced by human natural killer cells but not T cells during *Staphylococcus aureus* stimulation. *Infect Immun* 1993; **61**:3117–22.
- 186 Small CL, McCormick S, Gill N *et al.* NK cells play a critical protective role in host defense against acute extracellular *Staphylococcus aureus* bacterial infection in the lung. *J Immunol* 2008; **180**:5558–68.
- 187 Reading PC, Whitney PG, Barr DP, Smyth MJ, Brooks AG. NK cells contribute to the early clearance of HSV-1 from the lung but cannot control replication in the central nervous system following intranasal infection. *Eur J Immunol* 2006; **36**:897–905.
- 188 Reading PC, Whitney PG, Barr DP, Wojtasiak M, Mintern JD, Waithman J, Brooks AG. IL-18, but not IL-12, regulates NK cell activity following intranasal herpes simplex virus type 1 infection. *J Immunol* 2007; **179**:3214–21.
- 189 Pribul PK, Harker J, Wang B, Wang H, Tregoning JS, Schwarze J, Openshaw PJ. Alveolar macrophages are a major determinant of early responses to viral lung infection but do not influence subsequent disease development. *J Virol* 2008; **82**:4441–8.
- 190 Moore ML, Chi MH, Goleniewska K, Durbin JE, Peebles RS Jr. Differential regulation of GM1 and asialo-GM1 expression by T cells and natural killer (NK) cells in respiratory syncytial virus infection. *Viral Immunol* 2008; **21**:327–39.